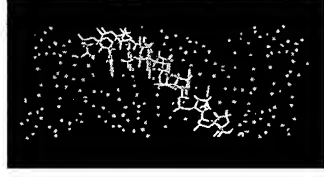


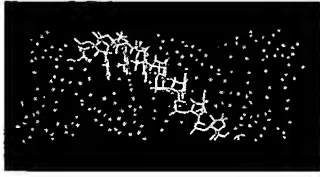
VIRAL RNA TARGET ENRICHMENT STRATEGY

By way of:



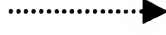
Triplex Formation

Figure 1



Triplex Formation

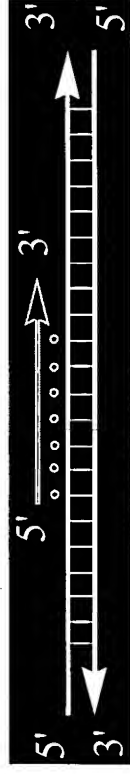
- **TARGET: POLYPYRIMIDINE STRAND**
- **PARALLEL-STRANDED HAIRPINS (HAIRPIN PROBES)**
- **CONTAINING 8-AMINOPURINES**



HIGHLY STABLE TRIPLEX STRUCTURES

Figure 2

TRIPLE HELICES FORMED by PARALLEL-STRANDED HAIRPINS



TARGET: POLYPYRIMIDINE STRAND

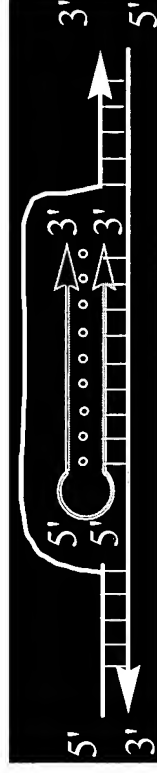
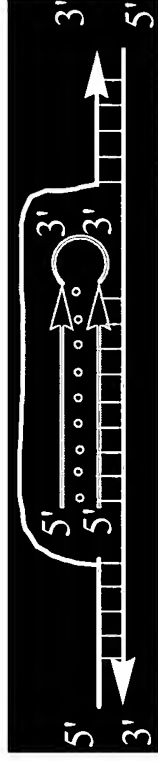
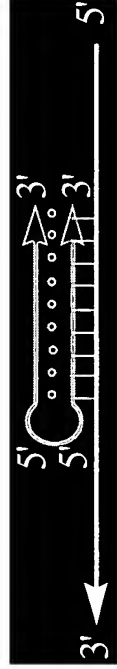
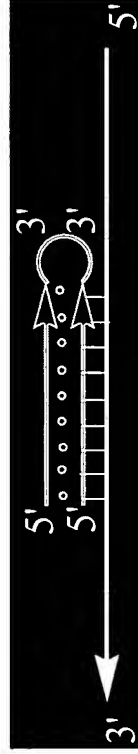


Figure 3

SYNTHESIS OF PARALLEL HAIRPINS

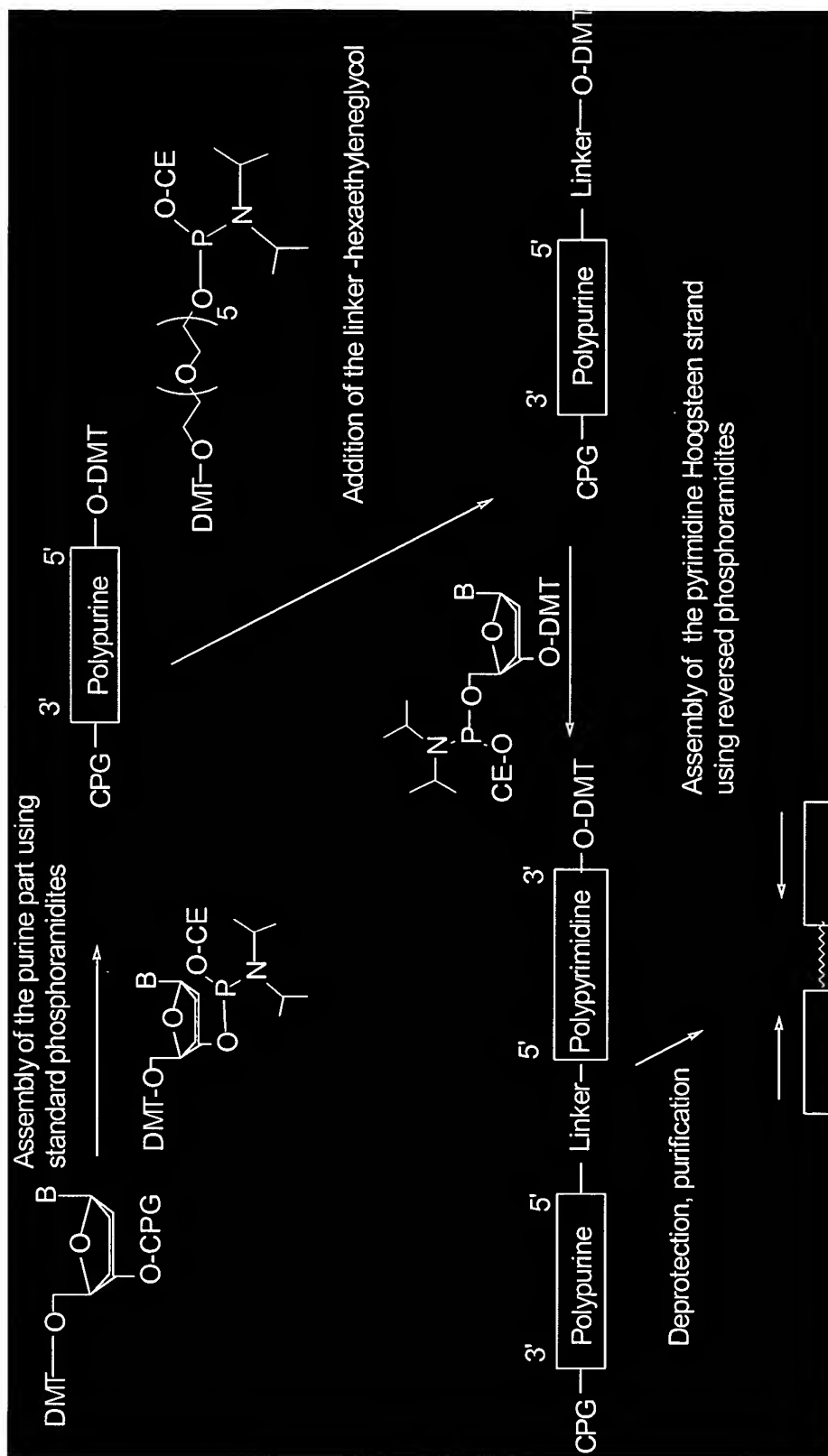


Figure 4

Synthesis of parallel-hairpins using asymmetric branching units

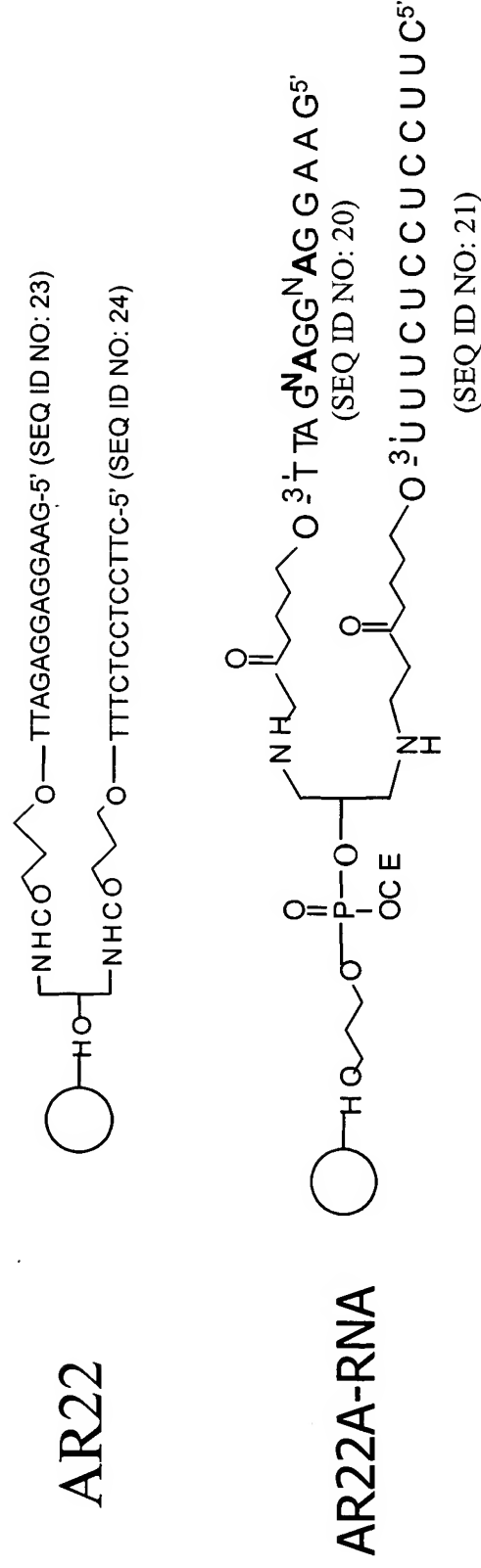


Figure 5

WNV INFECTED SAMPLES

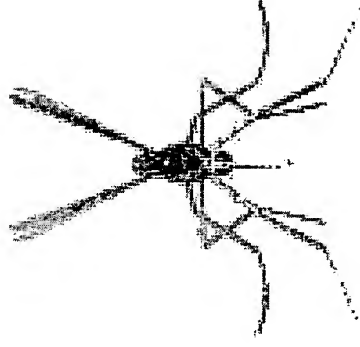
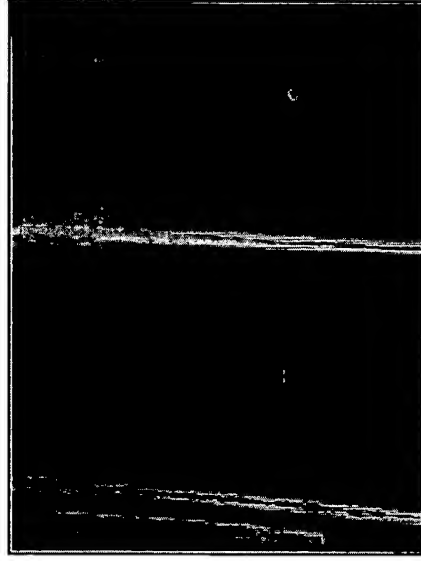
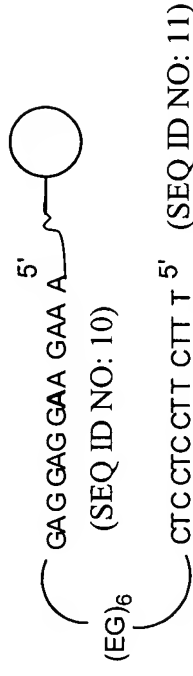
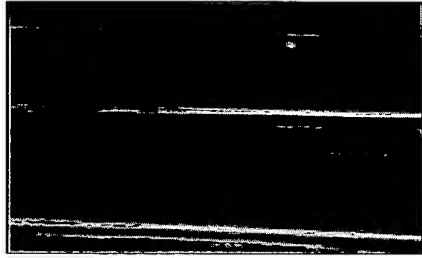


Figure 6

Strategy



WNV Hairpin Probe



RNA Extraction



RNA CAPTURE



**West Nile Virus
Infected Blood**

RNA Detection

Figure 7

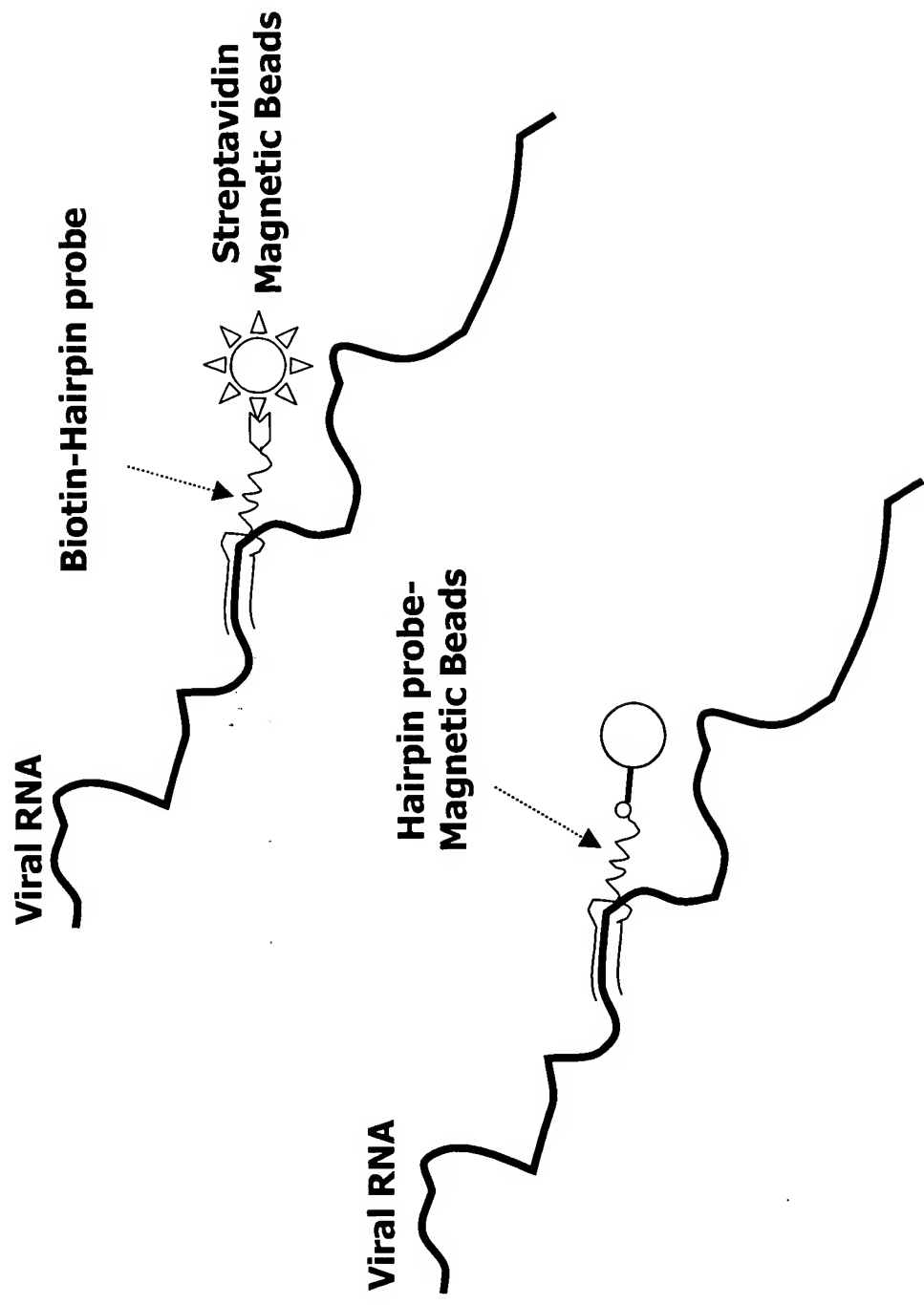


Figure 8

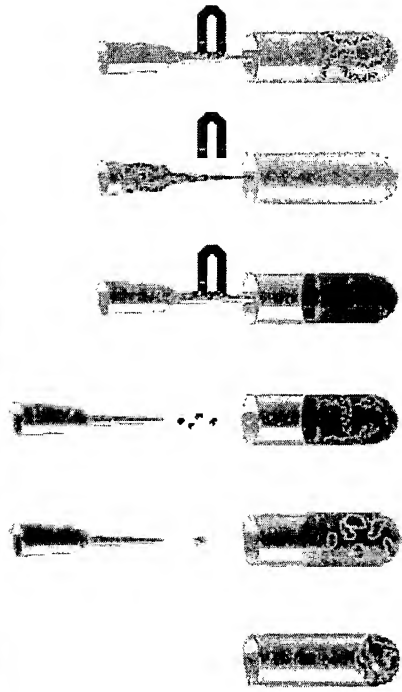
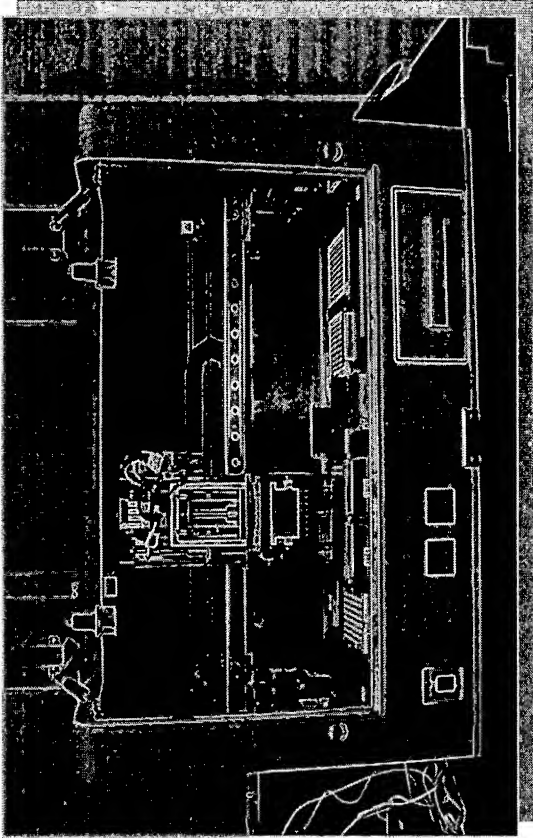


Figure 9

PYRIMIDINE MOTIF TRIPLEXES

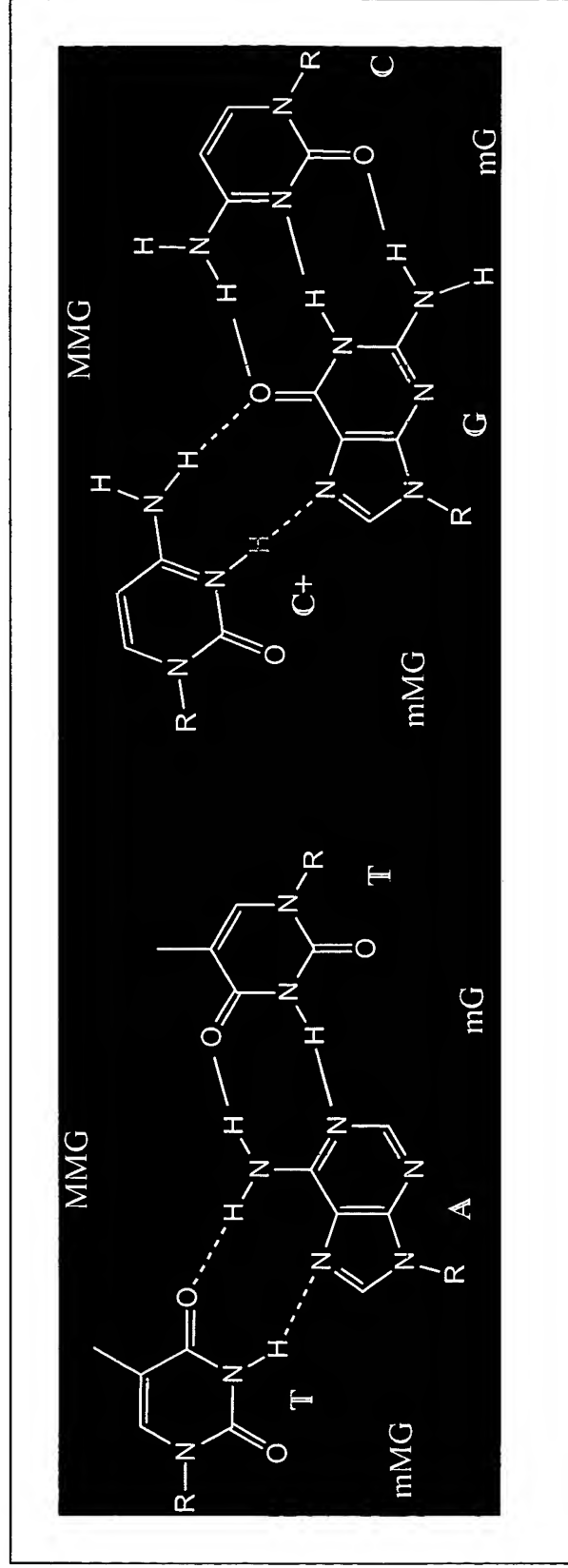
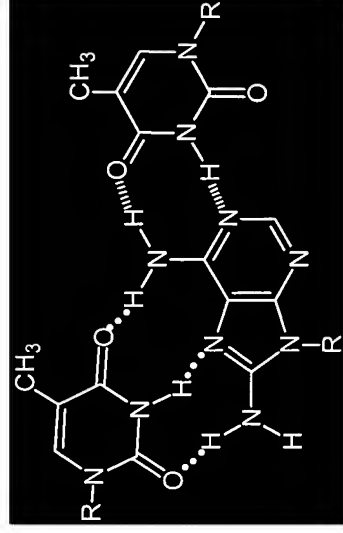
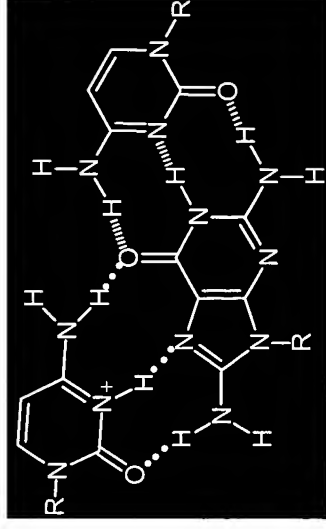


Figure 10

8-aminopurine derivatives

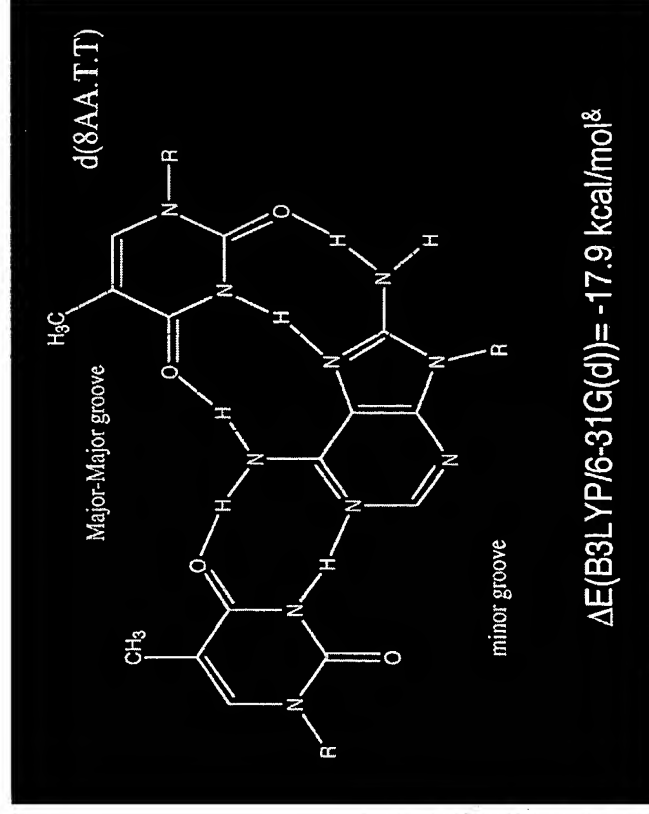
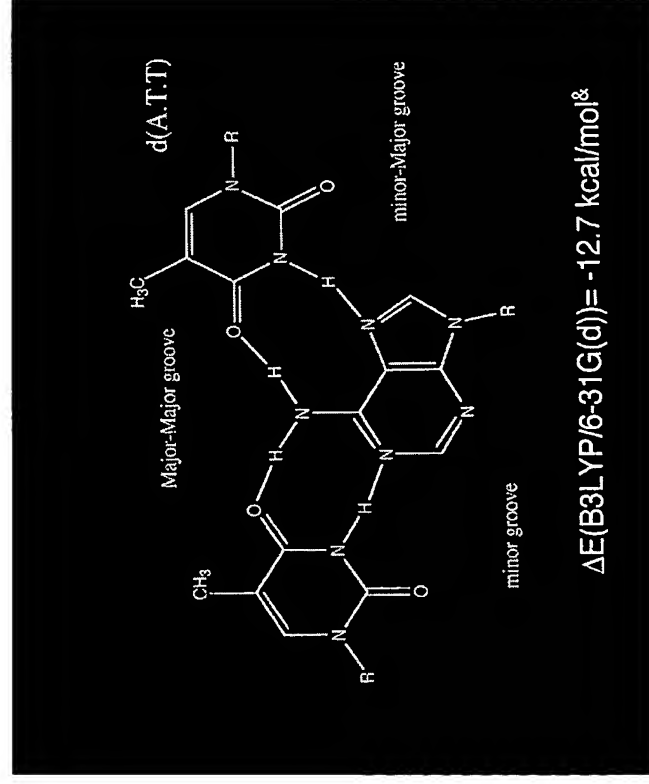


T·8-aminoA:T



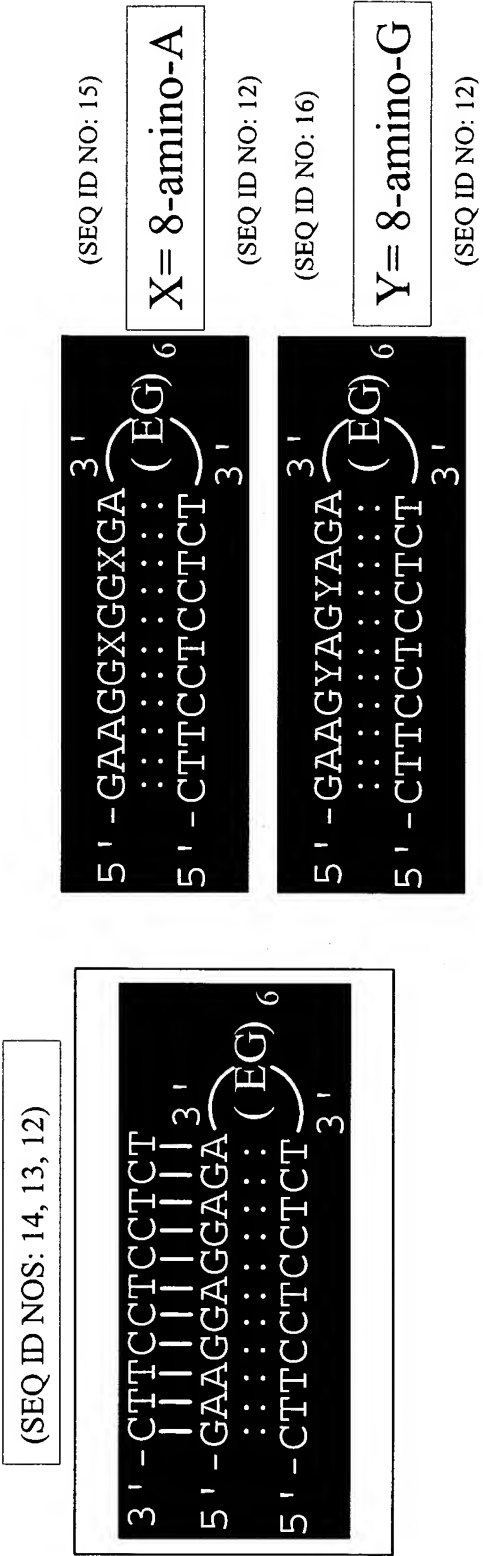
C⁺·8-aminoG:C

Figure 11



The 8-NH₂ group stabilize 5 kcal/mol the Hoogsteen pair

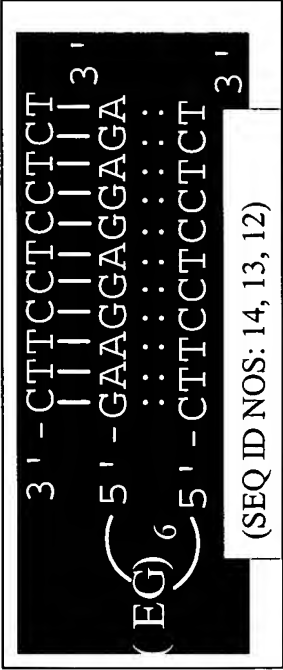
Figure 12



Melting temperature (°C) of triplexes [3'-3' hairpins]

Hairpin	pH 5.5	pH 6.0	pH 6.5	pH 7.0
Unmod.	56	47	36	32
8-aA	62	56	48	46
8-aG	67	59	53	51

Figure 13



Melting temperatures (°C) of triplexes [5'-5' hairpins]

Hairpin	pH 5.5	pH 6.0	pH 6.5	pH 7.0
Unmod.	54	45	33	20
8-aA	57	51	43	34
8-aG	69	59	50	40

Figure 14

Presence of one guanine at the polypyrimidine track

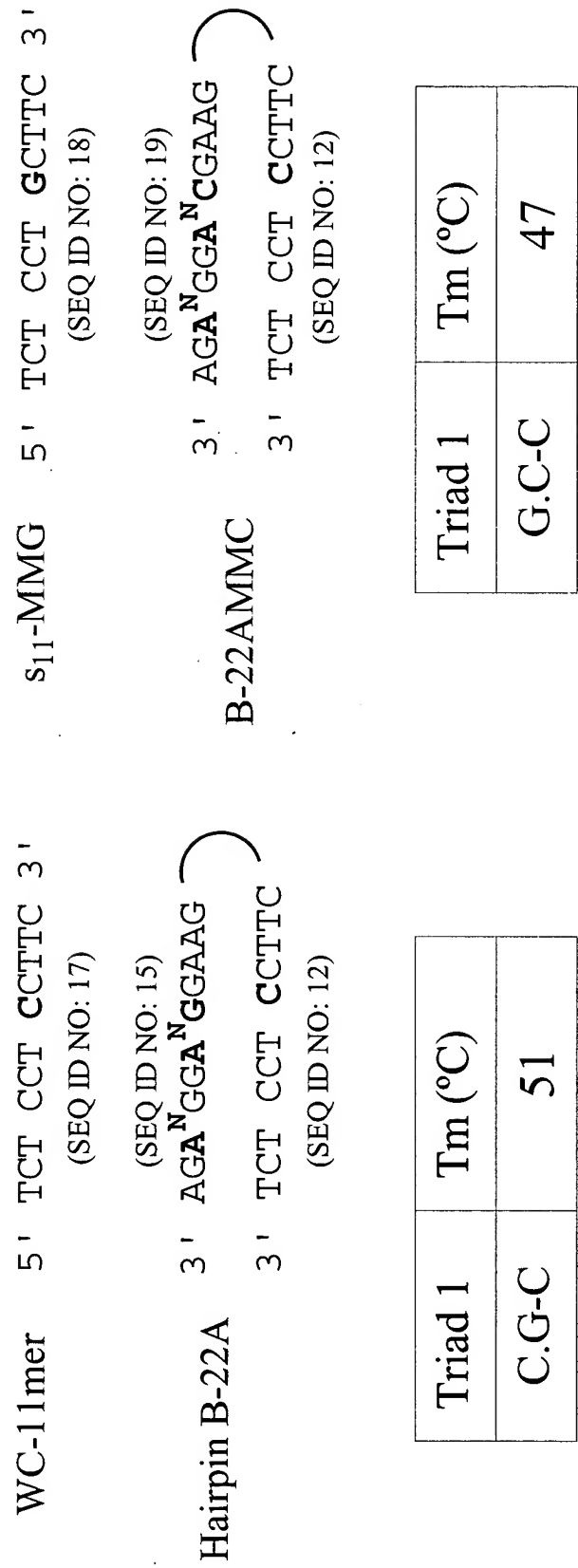
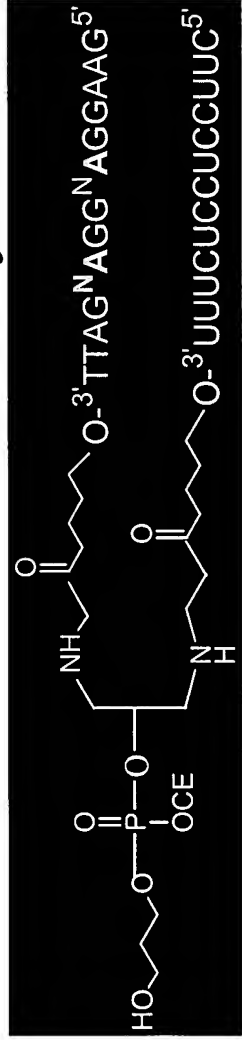


Figure 15

Melting temperatures (°C) of triplexes containing 2'-O-methyl-RNA



(SEQ ID NO: 20)

AR22A-RNA

(SEQ ID NO: 21)

5'UCUCCUCCUUC^{3'}

(SEQ ID NO: 22)

11-Me-RNA

Hairpin	Target 11-Me-RNA
AR22A-RNA	71

Figure 16

CD and NMR confirm that parallel duplexes are Hoogsteen

(SEQ ID NO: 15, 12)

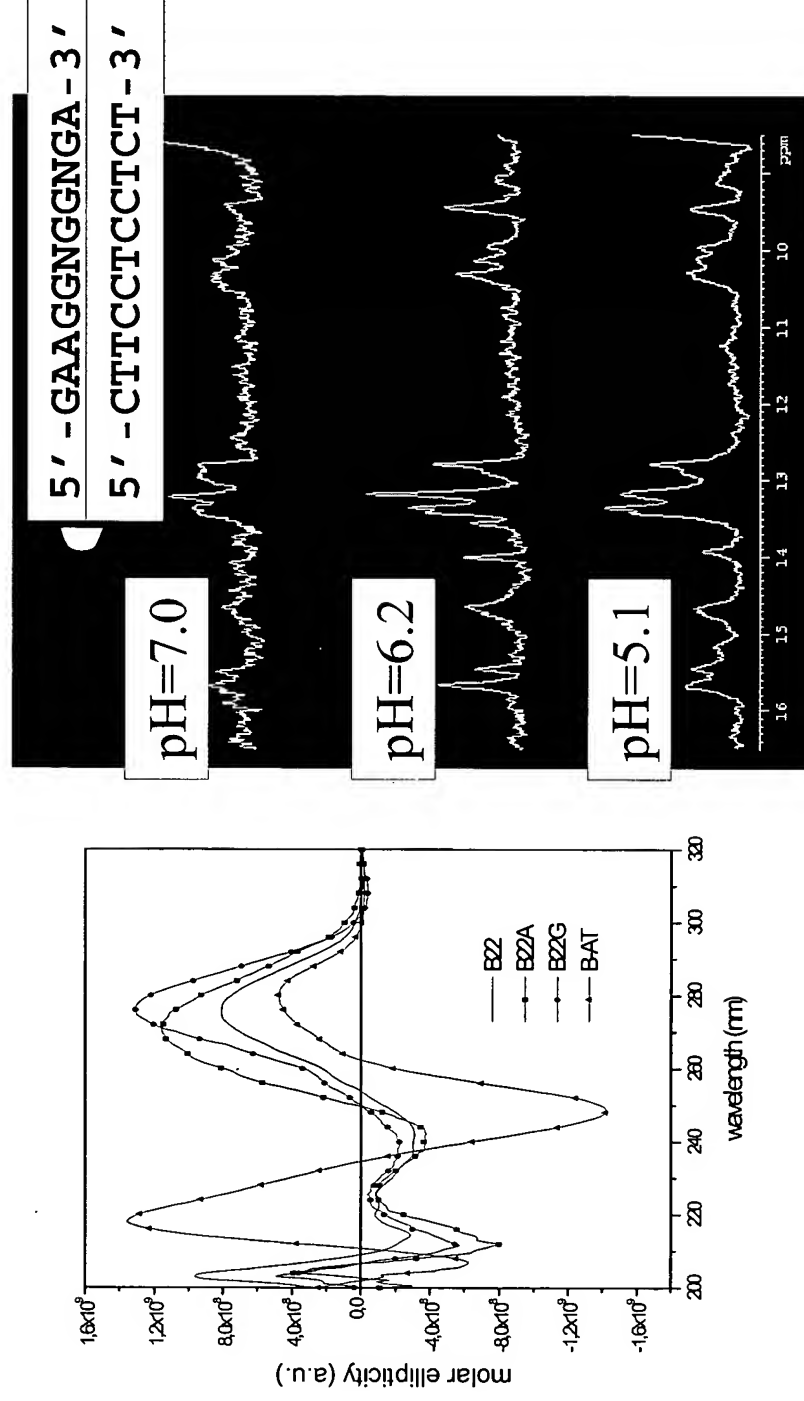


Figure 17

Gel-shift and NMR experiments confirm triplex formation

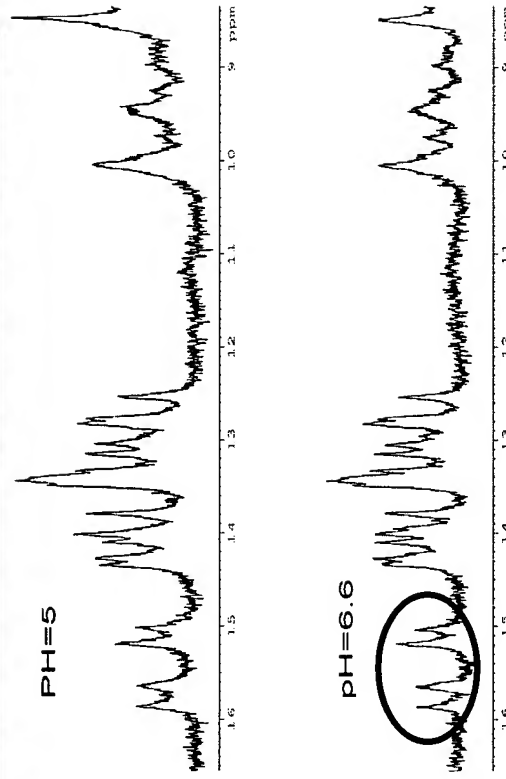
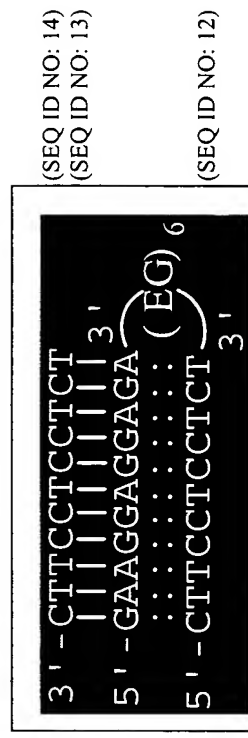
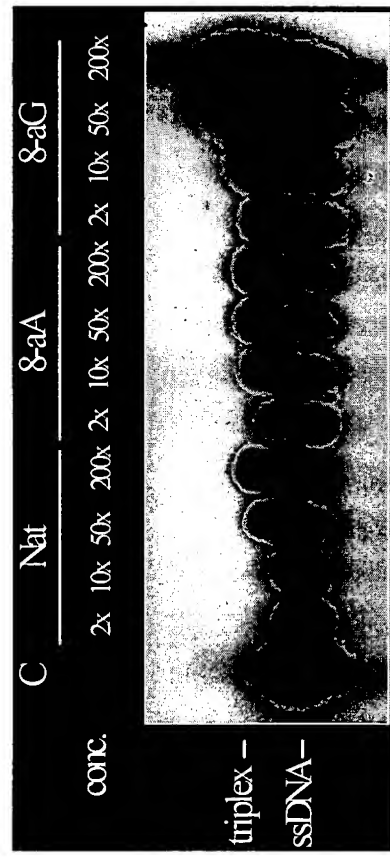


Figure 18




LOCUS
AF404756
11029 bp ss-RNA
linear
VRL 23-JUL-2002

DEFINITION
West Nile virus isolate WN NY 2000-crow3356, complete genome.

ACCESSION
AF404756

VERSION
AF404756.1
GI:21929238

SOURCE
West Nile virus.

Base Position	Sequence Target for Hairpin Design	
2043	CCCTTTTGTTC A	(SEQ ID NO: 7)
2443	CTCTTCCTCTCCGT	(SEQ ID NO: 2)
3857	CTGTTTTCTTTCA	(SEQ ID NO: 8)
6670	TTCTTCCTCCTCATGC	(SEQ ID NO: 3)
6740	CCTTTTCTGTT	(SEQ ID NO: 9)
6793*	CTGCTCTCCCTTCTCTT	(SEQ ID NO: 1)
7170	CTTCCCCCTTCGTC	(SEQ ID NO: 6)
7262	CACTCCTTTTITGCC	(SEQ ID NO: 5)
9727	CTCCACTTCCTCAAT	(SEQ ID NO: 4)

Figure 19

Effect of the Hoogsteen strand

Hairpin	Target	pH 5.5, 1 M NaCl	
		T _m (°C)	Hyperchromicity
B-22Acont (SEQ ID NOS: 15, 25)	WC-11mer (SEQ ID NO: 14)	41	+ 12 % (duplex to ss)
B-22Acont (SEQ ID NOS: 15, 25)	none	No transition	
B-22A (SEQ ID NOS: 15, 12)	WC-11mer (SEQ ID NO: 14)	57	+ 22 % (triplex to ss)
B-22A (SEQ ID NOS: 15, 12)	none	47	+ 12 % (duplex to ss)

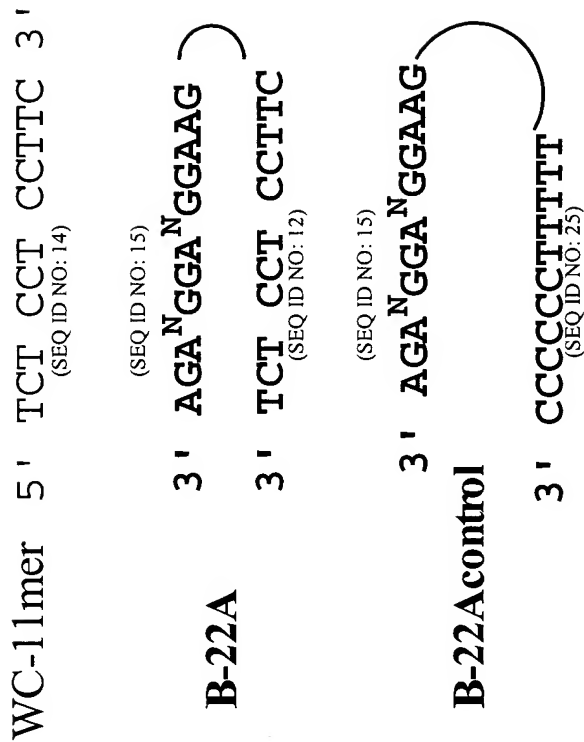


Figure 20